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BIOLOGY OF *NEOCHILDA FUSCA* N. GEN., N. SP. FROM THE NORTHEASTERN COAST OF THE UNITED STATES (PLATYHELMINTHES: TURBELLARIA)

LOUISE BUSH

Department of Zoology, Drew University, Madison, New Jersey 07940

A dark brown acoel has been collected for a number of years in bottom mud samples from Great Harbor and Buzzards Bay near Woods Hole, Massachusetts for use in class and experimental work. In the summer of 1965 I found a large population of the same species living intertidally in the salt marsh bordering Barnstable Harbor near West Dennis, Massachusetts. Since that time I have collected this species at a number of locations on the East Coast from Portsmouth, New Hampshire to Sandy Hook, New Jersey. Because of the presence of two distinct and conspicuous male reproductive organs at the posterior end of the animals it was natural for investigators to equate this species with *Childia spinosa* which was collected and described by Graff during his visit to Woods Hole in 1907 (Graff, 1911). Since *C. spinosa* has been shown to be the same species as *C. groenlandica* (Levinson, 1879) (see Hyman, 1959), several workers have recently published accounts using that designation (personal communication and Henley, 1968 and 1974; Costello, Henley and Ault, 1969; Boyer, 1971). However, a careful study of the anatomy of these brown acoels shows that they do not possess cuticular stylets as in *C. groenlandica* but, on the contrary, have eversible penes that preclude their being placed in the genus *Childia* at all. In fact it is necessary to set up a new genus and I am therefore describing them as *Neochildia fusca* n. gen., n. sp. and am presenting the data not previously published which I have obtained concerning their ecology and life history. It may also be noted here that, in spite of repeated efforts and extensive collecting, I have not been able to find specimens of *C. groenlandica* at the type locality (for *C. spinosa*) nor any place else along our coast, nor have I found anyone else who has seen it. It would be of great interest to obtain specimens that correspond to Graff's description for study.

Materials and Methods

Materials for this study were secured in two ways: (1) by taking samples of substrate in sand or muddy sand areas along marsh streams; (2) by grab sampling of muddy bottoms in bay areas near marshes. Samples were brought in to the lab and allowed to stand for several hours or overnight, by which time the animals would have come up to lie on the surface and could be picked up with a medicine dropper and transferred to small syracuse dishes together with a little of the substrate and the associated organisms to serve as food. Many specimens kept in such dishes survived for weeks although egg laying usually ceased after a few days. Young hatched from eggs laid in such cultures were raised for up to two months until the cultures had to be abandoned.

The life history was studied by making regular monthly collections at the West Dennis, Massachusetts salt marsh which was relatively easy of access throughout the year. Half-pint plastic containers were filled with the upper 1–2 cm. of sand along the stream where the animals were known to occur. The specimens were counted and measurements of length were made as they moved along in a large drop of water on a slide. Slight flattening of the animal by a coverslip after measuring allowed a determination of sexual condition and general maturity to be made. Collections in the winter had to be made by taking trash along the deeper parts of the stream where the animals had migrated from the sand. Many grab samples taken at various times as well as the experience of the supply department at the Marine Biological Laboratory gave additional data for comparison with that obtained at the marsh.

Many specimens were studied alive with ordinary light microscopy and with phase contrast. Most whole mounts and serial sections were prepared by flooding with Bouin's fixative at room temperature although some other methods were also employed for comparison, such as fixation with glutaraldehyde, freezing with CO_2, *etc.* Whole mounts were made of both stained and unstained animals both with and without bleaching. The most satisfactory stain for whole mounts was found to be either Grenacher's borax carmine or Riser's stain (Jones, 1966, page 245 F39, page 269 F177). Sections were made in paraffin at 8 μ and stained with hematoxylin and eosin, Delafield's hematoxylin and eosin, Mallory's triple stain or special stains for particular facets of the histology.

Observations, Results and Discussion

Systematic section

Neochildia n. gen.

The structure of the penes in *N. fusca* clearly distinguishes this species and makes it necessary to set up a new genus. The fact that the penes are inverted into the seminal vesicles as short straight tubes with some granular material at the proximal end is somewhat similar to the condition in several genera of the family *Convolutidae* as defined by Dörjes (1968), *e.g.*, *Pracaphanostoma*, *Pseudaphanostoma*, *Faerlea*. However, in the description of none of these do I find any mention of the crown of muscle fibers half way along the penes or of any similar mechanism by which the lower end of the penis is spread open to allow of the eversion of only the proximal half. The plesiomorphy here seems to me to be the eversibility of the penes which is characteristic of the Family *Convolutidae*: in the crown of muscles and type of eversion the genus *Neochildia* has evolved an apomorphic character distinctive for this genus. The two complete sets of copulatory organs constitute a good generic character, but the doubling of organs is not unusual in Turbellaria and does not have phylogenetic significance. The presence of unusually dark pigment may or may not be a genetic character. The definition of the new species must serve also as the definition of the genus until more species are discovered which belong here.

Neochildia fusca n. sp.

TYPES. Holotype, a longitudinal serial section from Buzzards Bay Massachusetts, United States National Museum (USNM) Cat. No. 52011; paratypes, whole mounts and serial sections from several localities, USNM Cat. Nos. 52012–52035 and in the author's collection.

DEFINITION. Shape elongated oval. Color dark brown with orange rhabdite packets. Length 4–6 mm. Brain internal with typical statocyst. Frontal organ not well developed. Mouth ventral without pharynx. Well developed body wall musculature with outer circular, diagonal, longitudinal and inner circular layers. Outer parenchyma dense and not vacuolated. Ovaries and testes paired, testes dorso-lateral to the ovaries and sometimes meeting in center behind the brain. Mature dark brown eggs lie dorsal and immediately posterior to the mouth. No female accessory organs. Two male copulatory organs opening separately into a shallow, ciliated atrium with genital pore slightly dorsal on the posterior end. Walls of copulatory organs thick, fibrous, with 10–12 longitudinal muscles spaced within the circular fibers. Penes eversible, with crown of small muscles attached half way along and acting, together with associated muscles in the parenchyma, to open the distal end of the penes when the proximal end is everted.

HABITAT. Mud or sandy mud at depths to 10 m all year; in summer it appears intertidally in brown, orange, or purple surface build-up on sand or sandy mud along salt marsh streams; in salinities from 2.2–35‰.

COLLECTIONS. Portsmouth, New Hampshire, intertidal sandy mud behind Wentworth Hotel each year in June 1968–72 and in August 1973; West Dennis, Massachusetts, in marsh stream north of Aquaculture Corporation Laboratory throughout the years 1965–74; also in Massachusetts in bottom mud of Buzzards Bay, in the Pocasset River, in marsh in Menemsha Harbor and in Provincetown Harbor, various times; New Jersey, Sandy Hook State Park Wildlife Sanctuary, Septembers 1966–70.

Description

COLOR. Medium orange brown in immature individuals to dark chocolate brown in adults.

SIZE. Newly hatched 0.2–0.35 mm; at first evidence of reproductive organs 1.2–1.3 mm; older animals to about 4.5 but a few to 6 mm.

SHAPE. Oval with broadly rounded anterior and slightly narrower rounded posterior; body thick to ~0.5 mm, with convex dorsal and slightly concave ventral sides; lateral edges at times somewhat enrolled. Often during spawning season the posterior 1/6 of the body may be suddenly narrowed, extended, and uptilted and then as briefly withdrawn. In this part the sperm filled seminal vesicles can be seen as two light colored ovals. For the significance of this see the discussion of reproduction and spawning.

STRUCTURES. Due to the very dark brown color many details cannot be distinguished in the living animals. In flattened animals with phase or light microscope the following can be made out (Fig. 1).

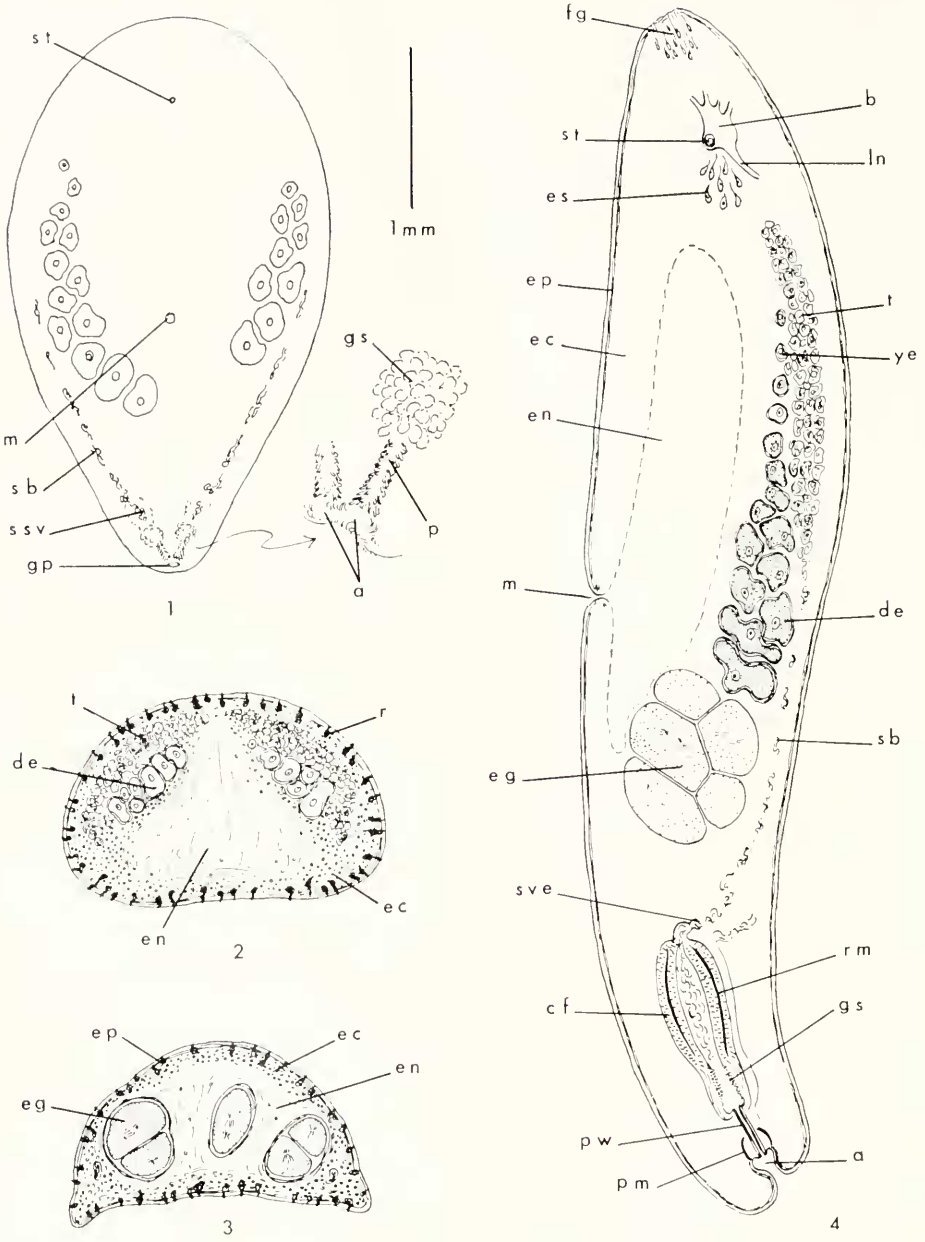


FIGURE 1. Living *Neochildia fusca*, slightly flattened. Abbreviations used are: a, atrium; b, brain; cf, circular fibers; de, developing eggs; e, epithelium, es, eosinophilic glands; ec, ectocytium; en, endocytium; ep, outer layers of body wall; fg, frontal gland; gs, granular secretion; gp, genital pore; p, penis; pm, protractor muscle; pw, penis wall; r, rhabdite gland; rm, retractor muscle; sb, sperm in bundles; ssv, sperm in seminal vesicle; st, statocyst; sve, seminal vesicle entrance; t, testis; ye, young eggs.

Cilia over the entire body; 5 μ long on dorsal side, 7–8 μ on lateral and ventral sides; isolated longer cilia scattered over body to 12 μ.

Color present both as dark brown granules in the outer epithelium and as orange color in solution and as orange rhabdite packets. Each rhabdite packet is made up of rice-grain-shaped 2.5 μ long bodies which may protrude on the surface as orange papules. Each packet is the product of a large gland cell the main part of which lies inside the epithelium. The glands and thus the associated papules are scattered at fairly regular intervals but are not arranged in regular rows. They are most numerous on the posterior third of the body on both dorsal and ventral sides. Over the rest of the body there are more on the dorsal than on the ventral side and at the very anterior end none occur in a small area where sections indicate there may be openings from a kind of frontal gland.

Mouth without a pharynx, lying about 3/5 of the way back from the anterior end.

Statocyst in the midline about 1/6 of the way from anterior end. Diameter in fixed mature specimens 25–30 μ. One statolith, a slightly flattened sphere.

Female reproductive system without accessory ducts or bursa. Developing eggs can be seen arranged in irregular rows down each side of the body with the largest just posterior to the level of the mouth. Eggs are dark brown and entolecithal.

Male reproductive system: in flattened animals during the spawning season mature sperm can be seen in light colored, coiled, irregular clumps extending from about the level of the largest eggs to the two male reproductive organs occupying the posterior 1/6 of the body. Seminal vesicles appear as light oval areas on each side with clusters of refractile granules and penes extending almost to the genital pore which is located slightly dorsal to the tip of the posterior end. Just inside the pore there is a shallow atrium into which the penes open separately. Testes are not to be distinguished clearly except in sections.

Findings from sections

Body layers. The epithelium consists of cells whose outer parts form a homogenous appearing surface layer and whose inner ends with the nuclei are insunk below and between the underlying muscle fibers. The homogenous layer (epicytium of some authors) (Hyman, 1959) contains brown pigment granules in a distinct outer band as well as scattered throughout the cell bodies. In some sections the band appears double with one line of granules near the surface at the base of the cilia and another deeper and thicker layer inside this. The epicytium is penetrated by the necks of the rhabdite gland cells, the bodies of which lie at various depths in the underlying parenchyma. In sections prepared with Bouin's fixative and hematoxylin stains the glands appear as empty spaces 10–15 μ across and 25–30 μ long. With glutaraldehyde fixation and Mallory's triple stain the contents appear as a bubbly irregular bright blue mass. In these sections

Figure 2. Cross section through region of developing eggs and testes. Abbreviations as in Figure 1.

Figure 3. Cross section through region of mature eggs. Abbreviations as in Figure 1.

Figure 4. Diagram of a sagittal section of entire animal. Abbrevations as in Figure 1.

the secretions can be seen to protrude at the surface to form the papules mentioned above. In sections cut horizontally near the surface of the body the necks of the glands appear as irregular circular spaces (3–5 in diameter) in the epicytium. In addition to these, minute "pin holes" (0.5 across) are arranged in irregular lines and must mark the outlines of the epithelial cells. Their appearance under high magnification suggests that they represent the part of the cell walls not firmly attached to each other.

Muscle fibers are well developed and occur both in the outer "body wall" and as large dorso-ventral strands running across the body. The body wall muscle occurs in four layers: (1) a sparse outer layer of circular fibers running between the inner ends of the epithelial cells; (2) a layer of two sets of diagonal fibers which cross each other at right angles and which are either interwoven or very closely associated; (3) the thickest and most conspicuous layer, relatively heavy longitudinal fibers; (4) inner circular fibers which are very sparse over most of the body but increase greatly in number in the posterior end of the body. Nuclei and epithelial cell bodies as well as the rhabdite glands lie within the muscle layers and, toward the inside, parenchyma cells also occur, making this entire outer wall a firm unit. Additional fine muscle fibers are arranged in concentric circles around the mouth so as to form an oral sphincter.

At the posterior end of the body the longitudinal fibers converge toward the genital pore. In this area also both the longitudinal and circular layers are increased in number so that there is a meshwork of fibers running perpendicular to each other and spaced at intervals throughout the parenchyma around the copulatory organs.

The ectocytium (peripheral mesenchyme of Hyman 1959) and the endocytium (central mesenchyme of Hyman 1959) are clearly distinguishable (Figs. 2, 3). The ectocytium is a parenchyma with rather closely spaced nuclei, forming a firm packing around the inner layer of the body wall musculature, various gland cells and the parts of the nervous and reproductive systems. The endocytium, as in most acoels, contains scattered nuclei, lightly staining irregular cytoplasm and vacuoles with partially digested food and storage granules.

Nervous system. The brain (Fig. 4) with the statocyst on its ventral side is located in the center of a cross section of the anterior end. Some eosinophilic gland cells occur in groups just posterior to the brain but their ducts, if any, could not be made out and the nerves leading from the brain are also difficult to distinguish in my sections. A large longitudinal posterior nerve proceeding dorsally on each side and at least two anterior nerves of good size are present.

At the anterior tip of the body certain cells in the parenchyma appear to have processes which run forward to penetrate the epithelium. The epithelium at this point is somewhat thinner, with shorter cilia and, as already noted, without rhabdite glands. These cells have nuclei similar to parenchyma cells, relatively small amounts of cytoplasm and only in certain sections do their processes (or ducts?) take any stain. Their exact nature has not been determined, but together they may be considered to form a sort of primitive frontal gland.

Female reproductive system. The first stages of egg development are found in the epicytium either a short distance behind the brain near the center or a

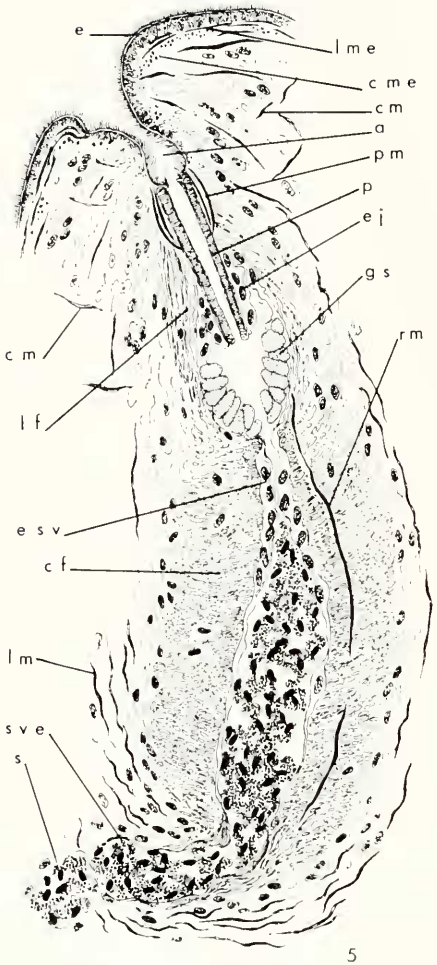
little to each side at this level. Cells which can be distinguished as eggs by their enlarged nuclei and conspicuous nucleoli occur singly. Progressively later stages extend posteriorly in a line along the body just medial to the line of developing sperm. The cytoplasm in the young eggs contains some brown pigment and as the cytoplasm increases in amount the pigment also increases until the newly laid eggs appear the same dark color as the adult animals. Developing eggs are surrounded by a layer of nurse cells which disappear by the time the eggs are ready to be laid. Eggs ready to be laid lie in a closely packed group pushed into the endocytium just behind the mouth (Fig. 3). Such eggs usually show mitotic figures, but the complete cytology of their development has not been worked out.

Male reproductive system. Developing sperm occur in follicles which are packed together in compact testes lying dorso-lateral to the eggs (Fig. 2). In actively reproducing animals the testes extend from a point where they meet in the middle of the body just behind the brain to a point just anterior to the level of the mouth on each side. At other times and in younger animals they begin further back on each side of the body. Large generative cells are found scattered throughout. The sperm in each follicle are surrounded by a thin membrane and remain together as they leave the testes and move down the side of the animal to enter the seminal vesicles.

My observations of living sperm agree with the descriptions presented by Henley (1968 and 1974). In sections the follicles with sperm are conspicuous not only because of the surrounding membrane and the uniform condition of the sperm in each one but also because of the refractile bodies present in the later stages. The refractile bodies also make conspicuous the presence of individual sperm scattered throughout the tissues of many animals during the spawning season. In some specimens also large masses of individual sperm are found, often located at the anterior end of the animal and lying either just inside of or partly on the surface of the epithelium. Such a mass shows no signs of membranes around groups of sperm and is larger than any single follicle. Often individual sperm appear to be moving out of the mass into the tissues. Clearly these masses as well as the individual sperm in the tissues are foreign sperm deposited on the surface of the animal by a partner.

The two copulatory organs lying side by side in the posterior end of the body are each a separate entity opening separately into the atrium (Fig. 5). The atrium itself is lined with a pigmented epithelium like that of the exterior except that the cilia are somewhat longer here. Since the two copulatory organs open into it from the anterior the atrium is broad anteriorly and it is short, narrowing down rapidly to the genital pore, so that it is actually, when at rest, rather like a fat heart with the point directed to the outside. When the penes are protruded the genital pore opens and turns outward so that the atrium is partly obliterated.

Each copulatory organ is pear-shaped with a short, curved, tube-like entrance extension at the anterio-lateral corner. The anterior and widest part of the organ proper comprises the seminal vesicle; the narrower posterior portion contains granular material and an eversible penis. The outer wall of the organ is composed of an epithelial lining and a thick (15–20 μ) layer of fibrous (muscular?) tissue, which is thickest around the seminal vesicle, and thinner around the penis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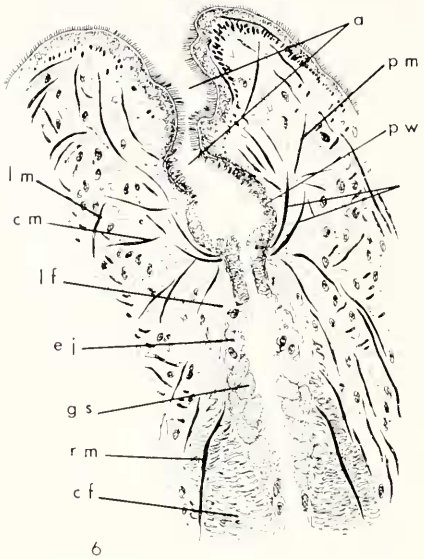
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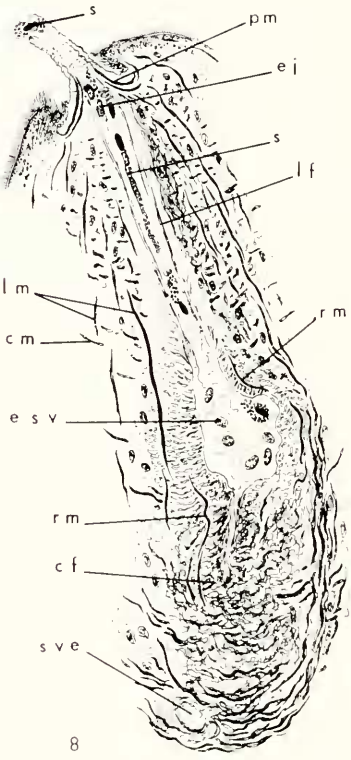
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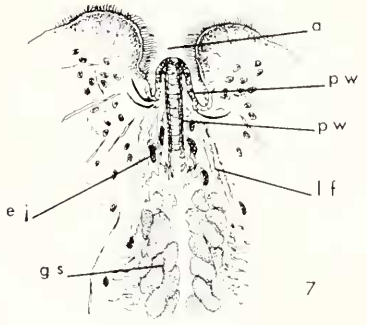
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and at the anterior end. Around the seminal vesicle the fibers run circularly but toward the distal end around the penis the direction changes so that the fibers run longitudinally. Here they are also finer. Around the entrance tube the circular fibers gradually decrease in number until they disappear and there is no distinct end to the tube clearly marking it off from spaces in the surrounding parenchyma. The fibers are distinctly different in staining reactions from muscles in the surrounding body and from the few muscles that form part of the wall. These latter are a series of widely spaced thick muscle fibers, ten to twelve in number, which run longitudinally through the circular fibers of the wall of the seminal vesicle. The question of the true nature of the fibers needs to be studied by histochemistry. There are only a few scattered nuclei present in the wall among the fibers.

The epithelial lining of the seminal vesicle is very thin with scattered nuclei, except for the area at the distal end where it is thicker with a number of large nuclei. Just beyond the nucleated area toward the penis a large amount of granular material is found, the granules of which are clumped in a cell-like arrangement although no nuclei could be found in the clumps. In cross section the appearance of this area suggests a thick cellular epithelium lining the organ distally as far as the penis. Since no granular gland cells can be found elsewhere it is suggested that the large epithelial cells at the end of the seminal vesicle act as holocrine glands which break down to form more granular material after ejaculation has used up that already present. Around the penis itself a few more nuclei indicate the presence of more epithelial cells, some of which can be found in the lining of the penis when it is everted. In the living animal the granular material is evident as a mass of glistening clumps at the proximal end of the penis.

The penis itself is composed of very thin fibers, a thicker inner circular layer and a very thin outer longitudinal layer, which arrangement is reversed, of course, when the penis is everted. The entire penis is straight, about 80–100 μ long; its most conspicuous feature is the presence of a crown of short muscle fibers (Figs. 5, 6, 7, 8, and see also Henley, 1974 Fig. 6B) which are attached about half way along the inverted penis and which run outward from there to a point at the proximal end of the male atrium. At this point a number of fine muscles of the ectocytium also attach and radiate outward toward the edges of the body. Sections showing penes in various stages of eversion indicate that this complex of muscles acts as protractor for the penis. The crown of muscles attached to the penis, on contracting, draws the penis outward at the same time as the radiating muscles in the ectocytium pull the lower end of the penis tube open so that the proximal half can be everted and pushed out through the genital pore by the forceful contraction of the circular muscles of the posterior end of the body.

FIGURE 5. Penis and seminal vesicle in the resting state. Abbreviations used are: a, atrium; cf, circular fibers; cm, circular muscle in parenchyma; cme, circular muscle in body wall; ej, epithelium of ejaculatory duct; esv, epithelium of seminal vesicle; gs, granular secretion; lf, longitudinal fibers; lm, longitudinal muscle in parenchyma; p, penis; pm, protractor muscle; pw, penis wall; rm, retractor muscle; s, sperm; sed, sperm in ejaculatory duct; sve, seminal vesicle entrance.

FIGURES 6 and 7. Stages in eversion of penis. Abbreviations as in Figure 5.

FIGURE 8. Completely everted penis with empty seminal vesicle. Abbreviations as in Figure 5.

This squeezing action would also collapse the seminal vesicle and force the sperm out of the body. The longitudinal muscles in the seminal vesicle wall plus the effect of relaxation of circular and contraction of longitudinal muscles in the posterior end of the body would act as retractors. The sudden narrowing and extension of the posterior end of the body during spawning corresponds with this sort of muscle activity.

Eggs and young. Egg masses measure about 1 mm in diameter and each contains from 7 to 40 eggs, each about 150 μ in diameter. Each egg is surrounded by a thin, tough, clear layer of jelly and the entire mass is further enclosed in a layer of sticky jelly, somewhat irregular on the outside and adhering tightly to the substrate though there is no formed stalk or attachment area. In the laboratory at 20° C development to hatching requires about 7 days. Newly hatched animals are oval in shape and have a statocyst and an outer layer of ciliated epithelium, but the parenchyma is not yet differentiated clearly into ectocytium and endocytium and there are no traces of reproductive organs.

Life history

A population of *N. fusca* in the marsh at West Dennis, Massachusetts was studied extensively because the area was easy of access and, being intertidal, allowed observations of the exact conditions under which the animals were living. Regular monthly collections were made in 1965–1966 and at less regular intervals for the next 8 years. Observations of the life cycles of other populations, particularly the collections made by grab sampler in Buzzards Bay correlate with the observations made at Dennis although there may be some minor variations in the time of events. The population in Buzzards Bay appears to be in all respects like that in the Dennis marsh.

The marsh at Dennis is built up on a sandy base between rows of sand dunes and the small stream which drains the marsh at low tide is completely under water at high tide. There is no fresh water runoff except immediately after a rain and the water in the stream measures at other times about 35‰. The upper part of the stream is formed by several meandering water courses and these, as well as the greater part of the main stream, are bordered by 1′–2′ high banks held in place by the roots of *Spartina* sp. which form a tangled mass collecting debris and fine silt in the water along the edges. The bottom of the stream is sand covered by a layer of fine brown sediment. Along the lower part of the stream it is wider with sloping banks that are uncovered but never completely dry at low tide. There is often a brown or purplish bacterial scum on the surface and in this and in the upper 2–3 cm of the sand bacteria, algae and a host of microscopic species including *N. fusca* are found in abundance. Similar locations in New Jersey, Martha's Vineyard, and New Hampshire have also yielded collections of this species.

In winter this exposed sandy area becomes completely barren of life. Several feet of ice may form on the vegetation away from the stream but my experience was that the stream itself remained open with only a thin film of ice on the surface. And in among the roots and trash in the water, although they were seldom found there in the summer, *N. fusca* was collected from December to April and early

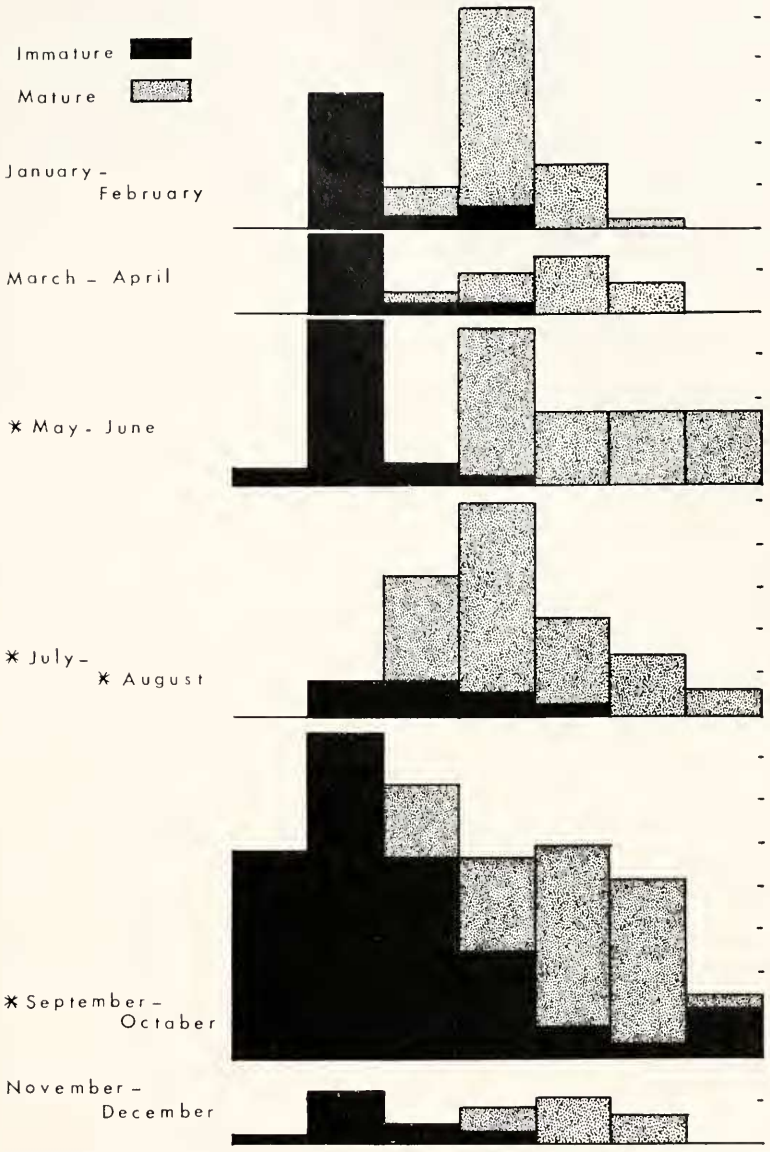

FIGURE 9. Annual life cycle of *Neochildia fusca*. The columns from left to right represent size groups: newly hatched —0.5 mm, 0.5–1.0 mm, 1.0–1.5 mm, 1.5–2.0 mm, 2.0–2.5 mm, 2.5 and above. Asterisk indicates spawning.

May. By the middle of May, when the summer populations were beginning to build up on the sand flats, *N. fusca* moved out from the stream and was to be collected in the sand. And in the middle of May the first animals were collected which laid eggs about 48 hours after being brought into the laboratory.

Figure 9 summarizes the annual cycle of the population at Dennis. Animals collected in pint samples of the top 2–3 cm of sand were measured and examined to determine their reproductive condition, and counted as belonging to the size groups in the graph. The population during the winter months, November to May, is made up of two groups: one, animals which go into the winter as adults and may have spawned already (the presence in the fall of sperm in the seminal vesicles and of young eggs suggests this); two, young animals which have hatched from the previous summer's spawning and are not yet mature. In May the members of the mature group laid eggs in the laboratory as noted and in the field newly hatched young began to appear in the collections in early June, which indicates that these older overwintering adults spawn at about the time when they move out of the stream and up into the sand flat. Shortly after this they evidently die off since I found that in early June spawning adults were very few in number, only a few, mostly spent, larger individuals being present. In the meantime the younger overwintering animals are maturing, so that by July and August they begin to spawn and many reproducing individuals are then present as well as newly hatched and young animals. Spawning continues into September, but with the onset of lower temperatures and shorter days further growth stops and the population, now made up of mature animals from the first spawning in May plus some from later spawnings who have matured and the young of all ages, move up into the stream to spend the winter in the debris at the edge of the pools there. Thus we have an annual life cycle with some animals probably spawning both in their first summer and then again the next spring before dying.

Behavior

Behavior of the animals when kept in small syracuse dishes in the laboratory was studied for up to two months after hatching as well as in individuals brought into the laboratory from time to time. Food was supplied by adding organisms from the collections in the field. Although activities observed in this way are not necessarily the same as they would have been in the field, they nevertheless indicate some things of interest.

N. fusca, although not a typically elongate interstitial animal, is nevertheless able to move through the substrate without difficulty as is attested by the fact that they come up to the surface of a bucket of mud or a dish of sand brought into the laboratory within a few hours. Their thick but flexible body with well developed body wall muscles and relatively solid parenchyma makes them seem much less fragile than many acoels. They move rapidly but smoothly and, on occasion as in feeding, can react very rapidly.

Feeding behavior is characteristic. Often in sand samples allowed to sit in the laboratory they were observed with the posterior end down between the sand grains and the anterior end raised slightly above the surroundings with the sides of the body slightly enrolled. Such a position makes them a perfect trap for passing animals. In a culture dish with less sand they lie quietly on the bottom, but in either case when potential prey appears a startling, quick, raising and then clamping down of the anterior end serves to pin down and hold the victim fast as it is pushed and sucked up through the mouth. Small crustacea, mostly copepods, as well as other turbellaria, nematodes and small annelids have all been found in the endocytium of mature animals. Animals up to about 1 mm long

usually contain only diatoms, perhaps because of size, but also it may be because of inability on the part of the young animals to capture active prey.

Cannibalism may occur in crowded cultures or in ones without sufficient food, but an avoidance reaction on the part of the animals probably prevents this under normal conditions. Whenever one *N. fusca* runs head on into another *N. fusca* there is an immediate and sharp withdrawal followed by a change of direction and moving away. Sensory structures at the anterior end undoubtedly are effective here although, as pointed out before, a distinct frontal organ is not evident in this area. This reaction has obvious advantages in preventing cannibalism and its suppression during mating is striking.

Besides the suppression of the avoidance reaction the characteristic quick outward thrust of the posterior end also marks mating behavior. Two animals will be seen to meet and instead of jerking away from each other, to spend some time moving about over each other in an exploratory fashion. On one occasion I was able to observe this for some time and I noted that finally one of the pair of animals turned slightly away from the other, shot out the posterior end so as to touch the partner quickly and lightly and then moved away. The two animals were immediately fixed and sectioned and one animal of the pair shows in the sections both penes protruded from the genital pore and the seminal vesicles emptied of sperm. Similar reactions were also observed on other occasions. This behavior together with the patches of sperm on or in the epithelium of many animals, plus the fact that the penis is a thin walled soft-appearing organ leads me to the conclusion that impregnation is by means of a deposition of sperm on the surface. The glandular material associated with the penis and extruded with the sperm may well have an enzyme which aids the sperm in penetrating the epithelium.

Egg laying was not observed satisfactorily. The presence of light appears to inhibit this and some workers have found that a sure way of obtaining eggs is to place the animals for several hours in a dark place. My attempts to observe the process in the cultures only resulted in the finding that the egg masses were usually deposited very early in the morning. Early one morning, however, when the lights were turned on to start observations I observed one animal to be moving around and around in a tight circle in one spot in an unusual way. This kept up for about 10 minutes and when the animal moved away it left behind a small egg mass on that spot. The position of the mature eggs in the endocytium just dorsal to the mouth make it seem logical that they would exit through that orifice, although it is not impossible that they would be extruded elsewhere. The fact that eggs are sometimes extruded through the mouth when animals contract on being fixed, the lack of any signs of torn epithelium in the animal observed above and the behavior of that animal leads me to expect the mouth to be the point of exit.

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Summary

1. *Neochildia* new genus is established on the basis of the presence of two separate and complete copulatory apparatuses, each with an eversible penis which has a crown of muscle fibers attached about half way along it. The type species, *Neochildia fusca*, although it has been used in a number of studies at the Marine Biological Laboratory and has previously been identified as *Childia groenlandica*, cannot be included in the genus *Childia* which is characterized by two copulatory organs but which is without any signs of eversible penes and has instead a bundle of cuticular stylets.

2. The anatomy and histology of *N. fusca* is described in detail. The most conspicuous field characteristic is the dark brown color which is unusual among acoels, and, indeed, among smaller Turbellaria in general.

3. The habitat of the new species is sand or sandy mud along marsh streams or in the bottom of associated shallow bays. The known distribution is from New Jersey to Maine along the East Coast of the U. S.

4. The life history involves the following annual cycle: an overwintering of mature or nearly mature animals subtidally or in the deeper waters of the marsh streams, an early spawning in May or June followed by a die-off of the overwintering animals, the maturing and spawning of the next generation from July to September and the overwintering of animals derived apparently from both the summer generations.

5. The food of young animals consists almost entirely of diatoms, but after the first few weeks they become highly carnivorous and are found to contain mostly copepods and other crustacea as well as worms and other Turbellaria.

6. Mating behavior is described and the conclusion is reached that sperm are deposited as a packet on the surface of the partner, not hypodermically injected. The very short contact between animals, the soft nature of the penes and the presence of masses of sperm entering through the epidermis in sections support this conclusion. Some evidence is presented that suggests that eggs are laid through the mouth.

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